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14. ABSTRACT

The focus of this grant is to investigate immunoprofiles for serum antibodies to aberrant glycans in human and animal models of mesothelioma. This is accomplished using a one of a kind printed glycan array which is at NYU School of Medicine (NYUSoM). It is hoped that these experiments will allow us to diagnose and prognosticate mesothelioma more accurately in the future. We have been severely limited by our ability to start the human mesothelioma glycoprofiles as well as the animal profiles due to delivery and set up times for our one of a kind glycomics laboratory at NYUSoM. We summarize the situation in the progress report with the good news that we will be moving onwards in June with these studies.

15. SUBJECT TERMS
Malignant Mesothelioma; Glycan Array; Immunoprofiles; Robotic Arrayer

16. SECURITY CLASSIFICATION OF: U	**17. LIMITATION OF ABSTRACT: UU**	**18. NUMBER OF PAGE 24**	**19a. NAME OF RESPONSIBLE PERSON** USAMRCC

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INTRODUCTION

The overall goal of our investigations is to identify a serum anti-glycan antibodies (AGAs)-based immunosignature of human malignant mesothelioma (MM) that would allow for identification of individuals, including military personnel, at high-risk for MM due to their potential long-term exposure to a carcinogenic form of asbestos, in time for an effective early intervention. Since such an immunosignature and the accompanying serum AGA immunoprofile reflect overall health, and more specifically immune health status of a person, both parameters are likely to also provide an insight into biological factors contributing to a susceptibility to this malignancy.
This project is funded in order to investigate immunoprofiles of serum anti-glycan antibodies recognizing Mesothelioma-derived aberrant glycans in human subjects and in animal models of Mesothelioma. This is accomplished using a one of a kind printed glycan array (PGA), which was developed by us at the New York University School of Medicine (NYU SoM), and was expanded by an addition of 182 novel glycan probes, many of which are Mesothelioma-specific.

Here we report:
(i) the preliminary results from immunoprofiling serum specimens collected monthly throughout the entire course of this 14-months experiment as proposed in the **Specific Aim II A** of this project; and
(ii) an outline and preliminary results of the experiment proposed in the **Specific Aim II B**.

BODY

Specific Aim II A: Using a rat model of asbestos-induced MM, immunoprofile serum AGAs using PGA and define temporal changes in this immunoprofile as mesothelial carcinogenesis develops and progresses during the 13-month experiment. The study was carried out using three groups of Fischer 344 female rats: (i) 32 animals exposed to intraperitoneal (IP)-applied asbestos/crocidolite as MM-inducing agent; (ii) 32 animals exposed to silica fiber as an IP-applied control for asbestos/crocidolite exposure, and (iii) 8 animals, for a one-time sham saline IP injection – as a control for age-related changes in AGA immunoprofiles.

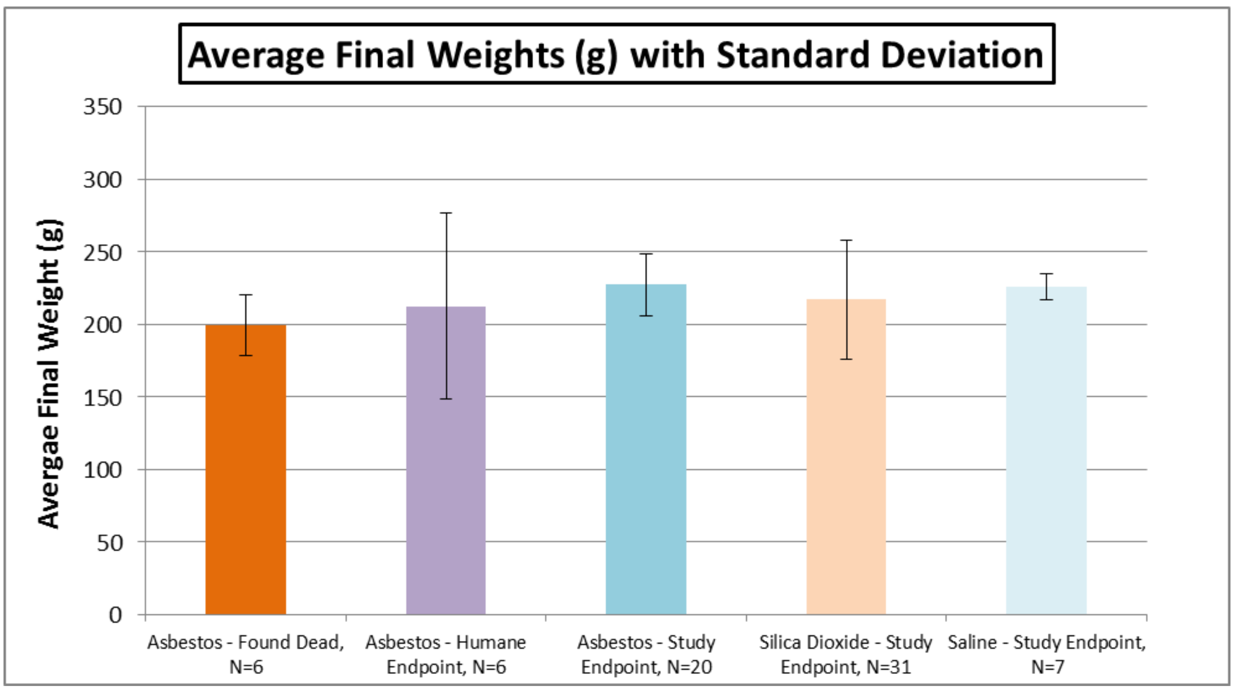
In the previous report, we presented a description of this longitudinal experiment and a figure showing the changes in weight over time for all animal groups. Inter-experimental follow-up procedures included (i) daily observations by the veterinarian staff, (ii) twice-weekly observations by the research associates participating in the project, and (iii) monthly weight measurements and blood collection. At the experimental end-point, animals were sacrificed according to the recommendations of IACUC. If animals exhibited symptoms of ill health, stress or fatigue prior to the study's endpoint, they were euthanized and necropsied before the study end-point at the "humane point".

End-point necropsy procedures included: detailed observations of the internal organs of each individual experimental animal, including photographic records of selected cases, and the collection of tumors and other tissues, including serum from each experimental animal. All observations made during the necropsy procedures were recorded and later transcribed into a full report, which is presented as **Attachment 1**. This document will also be included as an "Additional Dataset" in the report of this study, which is currently under preparation for publication.
Discussion of the preliminary findings:
1. There were variable responses to the peritoneally injected asbestos among the 32 experimental animals: 25 animals developed mesothelioma (78%), and 7 animals were found disease-free at the end-point necropsy.

Among the 25 animals that developed mesothelioma, 13 animals reached the study end-point: 7 animals had fully developed peritoneal mesothelioma, and 6 animals had minimal disease in the form of miliary tumors. Twelve animals did not reach study end-point: 6 animals were found dead of the disease and 6 were euthanized at the later stages of the experiment due to the animals' rapidly deteriorating health, which was the result of quickly progressing mesothelioma. This observation is very significant since it implies distinctly different individual responses to a carcinogen, similar to humans.

These different biological responses to a carcinogen are also indirectly manifested as differences in the weights of individual animals. In extreme cases, such differences are the result of "wasting" or the accumulation of large volume of ascites. This is reflected by large standard deviations in average final weight as shown in **Figure 1**, particularly in the group of asbestos-injected animals which had to be euthanized at the humane end-point.

Figure 1: Distribution of the body weight in the experimental animal groups at the study end-point or at the time of animal death due to the asbestos-induced mesothelioma progression. Based on the study outcome, asbestos-injected animals are further separated into the sub-groups of "Found dead of the disease, N=6", "Humane Endpoint, N=6" and "Study Endpoint, N=20".



2. During the experimental end-point necropsies out of 32 animals injected with silica, 31 animals were found disease-free and one animal was found to have developed a sarcoma tumor.

3. All control saline-injected animals were found disease-free at the experimental end-point necropsies, and control saline-injected animals and silica-injected animals survived until experimental end-point.

<u>Preliminary results of rat serum immunoprofiling are presented in Attachments 2 and 3.</u>

Attachment 2 shows three sets of bar-graphs presenting fluorescence intensities of rat serum anti-glycan antibodies binding to glycan probes present in our Printed Glycan Array NYU PGA-400. The top bar-graph shows pre-injection AGAs for all three experimental animal groups:

"asbestos", "silica" and "saline". In all three bar graphs, asbestos-injected animals are colored red, silica-injected animals are colored blue, and saline-injected animals are colored green. The middle bar-graph shows one-month post-injection AGAs for all three experimental groups, and the bottom bar-graphs shows AGAs at the study endpoint for all three experimental groups. The endpoint AGA immunoprofiles are obtained from the sera of individual animals, whereas the AGA "pre-injection" and "1 month post—injection" immunoprofiles are obtained from the pooled sera of three to five animals. Sera of these animals were pooled due to the low volume of blood collected from the tail vein of animals still young and small at early experimental time-points.

The bar-graphs have been aligned in a way that allows us to observe changes in the individual AGAs over time between the experimental animal groups. For instance, appearances of specific AGAs in response to the asbestos injection are detectable in the "1 month post-injection" immunoprofiles. Significant and distinct differences between serum AGAs intensities in "asbestos" vs. "silica" vs. "saline" rats at the study endpoint are also immediately noticeable.

Attachment 3 presents structures of selected glycans exhibiting distinct serum AGA binding patterns in "asbestos" vs. "silica" rats four weeks following injections. Antibodies against glycans marked by "**X**" show significant dynamics in response to asbestos exposure and during mesothelioma development in both rat and human populations.

We are preparing the results of this experiment for publication, which will be submitted once we complete rat serum immunoprofiling and analyses. This manuscript will be included in the final study report.

Specific Aim II B: Use the syngeneic II-45 cell line xenograft in rat model of asbestos-induced mesothelioma to correlate mesothelioma tumor growth with rat serum anti-glycan antibodies (AGA).

The goals of this experiment are: (i) to identify glycans showing the dynamics of anti-glycan antibodies during outgrowth of syngeneic mesothelioma tumors, implanted intraperitoneally (IP) or subcutaneously (SC), by comparing immunoprofiles of saline-injected control rats to rats with the implanted tumor cells, and (ii) to identify glycans showing the dynamics of anti-glycan antibodies in response to the chemotherapy drug Gemzar by comparing immunoprofiles of Gemzar-injected rats with the immunoprofiles of saline-injected control rats. Gemzar is an anti-cancer drug often used in MM treatment, and is known to have immunomodulatory effects. In this experiment we investigated whether this immunomodulatory effect is detectable on the level of the AGA dynamics in healthy animals.

Preparations for this study have been described in the previous report. Briefly, we have re-grown fresh stocks of syngeneic rat mesothelioma II-45 cells and performed testing for a panel of animal pathogens, with specific focus on rat pathogens. As determined by Charles River Research Animal Diagnostic Services, our II-45 cell line was pathogen-free, and was ready for injections as proposed in the second arm of the study.

Experimental Design and Schedule are shown in **Table I** below.

Table I: Schedule of an experiment performed under Specific Aim II B:

Group		6/13/14	6/27/14	7/1/14	7/11/14	7/15/14	7/24/14	7/25/14	7/29/14	7/31/14
		Day - 18	Day - 4	Day 0	Day 13	Day 14	Day 23	Day 24	Day 28	Day 30
Control	6 IP Females	Bleed	Bleed	Injection	Sacrifice: bleed	Bleed	Sacrifice: bleed	Bleed	Sacrifice: bleed	Sacrifice: bleed
	6 SC Females	Bleed	Bleed	Injection	Sacrifice: bleed	Bleed	Sacrifice: bleed	Bleed	Sacrifice: bleed	Sacrifice: bleed
II-45 Cell Lines	12 IP Females	Bleed	Bleed	Injection	Sacrifice: bleed & harvest tumors	Bleed	Sacrifice: bleed & harvest tumors	Bleed	Sacrifice: bleed & harvest tumors	NA
	12 SC Females	Bleed	Bleed	Injection	Sacrifice: bleed & harvest tumors	Bleed	Sacrifice: bleed & harvest tumors	Bleed	Sacrifice: bleed & harvest tumors	NA
Gemzar	12 TV Females	Bleed	Bleed	Injection	Sacrifice: bleed	Bleed	Sacrifice: bleed	Bleed	Sacrifice: bleed	Sacrifice: bleed

Prior to the first blood draw, 3-4 week old Fischer F344 female rats with tattooed tails featuring unique identifying numbers were acclimatized for 18 days. After 3 weeks of acclimatization, the experiment began. In accordance with NYUSM DLAR blood drawing policy, blood draws were performed by tail nicking in order to obtain 200-500 µL of blood per draw.

On day 0, $1x10^6$ syngeneic rat mesothelioma cells were injected into the rats' dorsal flanks in 0.2 mL of HBSS via a subcutaneous (N=12) or intraperitoneal (N=12) injection. Control animals were injected with 0.2 mL of HBSS via a subcutaneous (N=6) or intraperitoneal (N=6) injection. A 0.2 mL solution of Gemzar (40 mg/kG body weight) in HBSS was injected into "Gemzar" rats (N=12) via a tail vein.

Animals were observed daily by DLAR staff and at least two times per week by research associates participating in the project. Tumor growth was monitored over a 4 week period using a digital caliper.

To characterize an "early" stage of tumor growth, on day 13 tumors were removed from euthanized animals in the subcutaneous (SC) and intraperitoneal (IP) cell line group. Resected tumors were examined for their pathological features, and stored in formalin for further analysis.

For comparison, 6 animals from the subcutaneous (SC) and intraperitoneal (IP) control group, and 3 animals from the Gemzar group were also sacrificed on day 13. All animals were

sacrificed according to the recommendations of IACUC. At day 24 and day 28, tumors from the subcutaneous (SC) group and intraperitoneal (IP) cell line group were harvested and stored in a similar manner. The remaining control and Gemzar animals were sacrificed on day 30 at the conclusion of the experiment.
End-point necropsy procedures included: detailed observations of the internal organs of each individual experimental animal, including photographic records of selected cases, and the collection of tumors and other tissues, including serum from each experimental animal. All observations made during the necropsy procedures were recorded and later transcribed into a full report, which is presented as **Attachment 4.** This document will also be included as an "Additional Dataset" in the published report of this study.

AGA immunoprofiling of serum specimens is in progress.

Classification of rats implanted with mesothelioma syngeneic cells, N=24, based on the study outcome:

Disease free, study endpoint, N=0;

- Minimal disease, study endpoint, N=0;
- Fully developed disease, study endpoint, N=13;
  - SC: N=9;
  - IP: N=4;
- Fully developed disease, humane endpoint, N=7;
  - SC: N=3;
  - IP: N=4;
- Fully developed disease, found dead, N=4;
  - IP: N=4;
    - Found dead on 7/23/14, N=3:
    - Found dead on 7/28/14, N=1;

Main Observations from the Specific Aim II B animal experiment:

- Saline-injected rats: no health problems observed, no tumors found at the necropsy.
- Unusually fast growth of implanted Mesothelioma cultured cells noted after the death of:
  - 3 IP rats on day 22 of the study;
  - 1 IP rat on day 27 of study;
- Due to faster than expected growth of implanted Mesothelioma cultured cells, the experiment concluded faster than expected.

KEY RESEARCH ACCOMPLISHMENTS

1. We have further expanded the array platform, NYU-PGA-400 by adding 182 novel glycan probes, many of which are human Mesothelioma-specific. It is expected that this expanded glycochip will allow us to better diagnose and prognosticate Mesothelioma earlier during its development, and obtain larger volume of information about the pathobiology of the immune system under a pressure of the asbestos exposure and during the mesothelioma development.

2. We have generated a large library of rat anti-glycan immunoprofiles immunoprofiles obtained from the AGA immunoprofiling of sera collected periodically in the 13-month experiment following exposure of animals to asbestos – as a carcinogen, or to silica – as an irritant. This library of AGA longitudinal immunoprofiles is already showing a great value as a source of information allowing an insight into the (rat) immune system responses and into the AGA dynamics following exposure to environmental factors harmful also to the human health.

3. We have identified a set of glycans indicated by a dynamics of the immune response to asbestos in both rats and humans. Identification of the real targets of antibodies recognizing these glycans should help to develop the mesothelioma-preventive strategies. Such preventive strategies may include immune system-correcting / reconstituting intervention, as a single modality or as a companion-preventive therapeutics.

REPORTABLE OUTCOMES

Results presented here in all four attachments are components of publications currently under preparation. These publication(s) will be submitted once we complete rat serum immunoprofiling and analyses of both animal experiments. These manuscripts will be included in the final study report.

CONCLUSIONS

1. Experimental rats show distinctly different individual responses to both asbestos-environmental carcinogen, and silica – environmental irritant. This observation validates one of our initial working hypotheses and is very significant since it implies different individual immune responses to carcinogen(s) and irritant(s), similar to humans. Furthermore, this observation also confirms a suitability of Fisher 344 rat as a model-animal to explore mesothelioma-preventive intervention strategies.

2. Certain anti-glycan antibodies are induced or show marked dynamics of their concentration in circulation in response to the bolus - peritoneal injections of asbestos or silica; this dynamics of the “early response AGAs” is observed in the first serum collected four weeks following injections, it is more pronounced in some experimental animals than in the others, and it differs between asbestos-injected and silica-injected animals.

3. The dynamics of certain AGAs over time appears to correlate with the type of animal response to asbestos exposure: this is a preliminary observation - the final conclusion will be reached after completion of immunoprofiling of all longitudinally collected sera of “asbestos rats”.

4. It will be important to resolve the time-period of four weeks following injections of asbestos and silica, to capture earliest points of the “early response AGAs” generation and the presence in the circulation of exposed animals. It will also be important to determine an isotype of the “early response AGAs” and “response AGAs” to better understand immune system components and mechanisms involved in the dynamics of the response to asbestos exposure.

5. Serum antibodies against selected set of glycans show significant dynamics in response to asbestos exposure, and during mesothelioma development in both rats and humans. Identification of the real targets of antibodies recognizing these glycans should help to develop the mesothelioma-preventive strategies. Such preventive strategies may include immune system-correcting / reconstituting intervention, as a single modality or as a companion-preventive therapeutics.

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APPENDICES:

Attachment 1: Transcripts of the observations made during the necropsies in all three experimental groups of animals intraperitoneally (IP)-injected with asbestos/crocidolite, animals IP-injected with silica fiber, and animals sham-IP-injected with saline.

Attachment 2: Three sets of bar-graphs presenting fluorescence intensities of rat serum anti-glycan antibodies in all three experimental groups. The bar-graphs show AGAs in sera collected “pre-injection”, “one-month post-injection” and at the “study endpoint”.

Attachment 3: Structures of selected glycans exhibiting distinct serum AGA binding patterns in “asbestos” vs. “silica” rats four weeks following injections. Antibodies against glycans marked by “X” show significant dynamics in response to asbestos exposure and during mesothelioma development in both rat and human populations.

Attachment 4: Experimental schedule and notes from observations made during the necropsies of all groups of animals injected with the syngeneic cell line xenograft, saline, and Gemzar.

Award Number: W81XWH-10-0399 and W81XWH-10-400

TITLE: Glyco-Immune Diagnostic Signatures and Therapeutic Targets of Mesothelioma

PRINCIPAL INVESTIGATORS: Harvey Pass, MD; Margaret Huflejt, PhD

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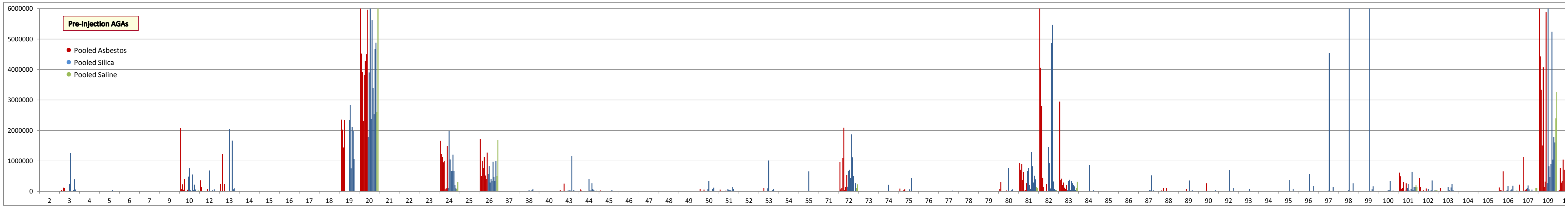
Attachment 1 of 4

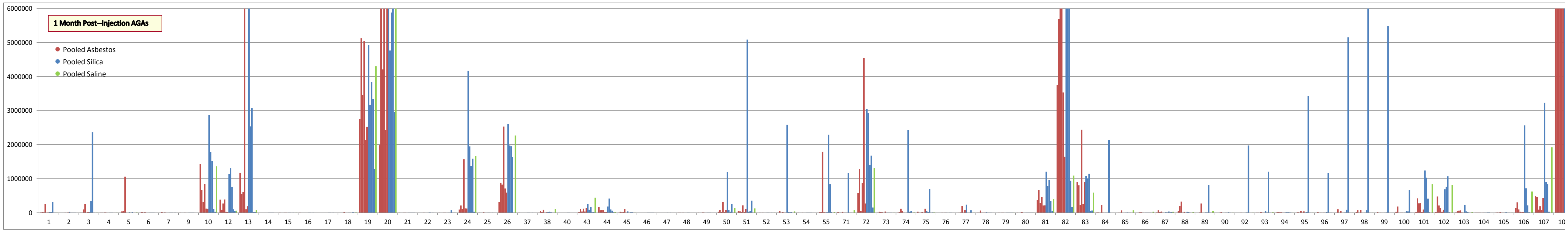
#	Rat #	Treatment	Endpoint	Final Wt.	Meso?	Necropsy Notes
Single Bolus Intraperitoneal Asbestos Injection						
1	3	Asbestos	Found Dead	197.5 g	Yes	1. Lots of Milliary Tumors on meso of abdominal cavity, tissue and small intestine 2. 20mL ascitic fluid collected
2	16	Asbestos	Found Dead	205 g	Yes	1. Milliary Tumors (.5 to 5mm)all over chest/abdomen and cirrohsial tumors 2. Lots of Ascitic Fluid 3. Liver adhered to diaphram 4. Black lymph nodes in abdominal peritoneum
3	55	Asbestos	Found Dead	210.8	Yes	1. Milliary Tumors (1-1.5mm)on intestines and reflected on peritoneum+ Coalescing Together 2. Ascitic Fluid 3. Liver/Spleen Adhered to Diaphram
4	60	Asbestos	Found Dead	223 g	Yes	1. 3cm Large tumor outside of Intestine, when cut pus/fluid came out 2. Small Milliary tumors all over abdominal wall 3. Still food in stomach 4.Liver/diaphram/stomach/spleen/pancreas/small intestine all adhered together
5	64	Asbestos	Found Dead	161.3 g	Yes	1. Very few (<10) milliary tumors 2. 1.5cmx8mm tumor adhering to intestine 3. 1 2cm x 1.4cm tumor adhering to intestines 4. very little ascitic fluid 5.adhesions of organs
6	66	Asbestos	Found Dead (<hours)	201.1 g	Yes	1. Milliary tumors all over organs, especially on intestine 2. 30mL of yellow ascitic fluid collected 3.Liver/diaphram/stomach/spleen/pancreas/small intestine all adhered together
7	43	Asbestos	Humane Endpoint	179.2 g	Yes	1. Many milliary tumors, mulitple nodules 2. Adhesions of liver/diaphram/stomach and pancreas/spleen 3. 26mL of clear asctic fluid 4. Digestive Tract still instact 5. Vascular still in tact
8	54	Asbestos	Humane Endpoint	194.6 g	Yes	1. Lots of Milliary Tumors 2. Tumors Small + Blue on the stomach 3. Possible 2-3cm Large tumor behind right kidney 4. 42mL ascitic fluid collected 5. Dipahram adhered to stomach/ribs
9	57	Asbestos	Humane Endpoint	180.3 g	Yes	1. Tumors on digestive tract, very few tumors reflected on abdomen lining 2. "Floating Meso", saved sample 3. 1x5mm tumor under spleen and 5x5 tumor lining large intestine 4. 1-2.5mm on intestines 5. high amount of tumors on retopreitoneum, kidney 6. 2.5mm tumors by bladder 7. 20mL ascitic fluid 8. Bloated intestines 9. Liver adhered to stomach/diaphram 10. Spleen adhered to stomach/pancreas/small int
10	59	Asbestos	Humane Endpoint	225 g	Yes	1. Many 1/2 cm tumors lining large intestine 2. 2cm large tumor w/own blood flow b/w liver and stomach 3. large tumor in right upper quadrant under right liver lobe 4. 42mL of Ascitic Fluid Collected 5. Liver adhered to diaphram/stomach

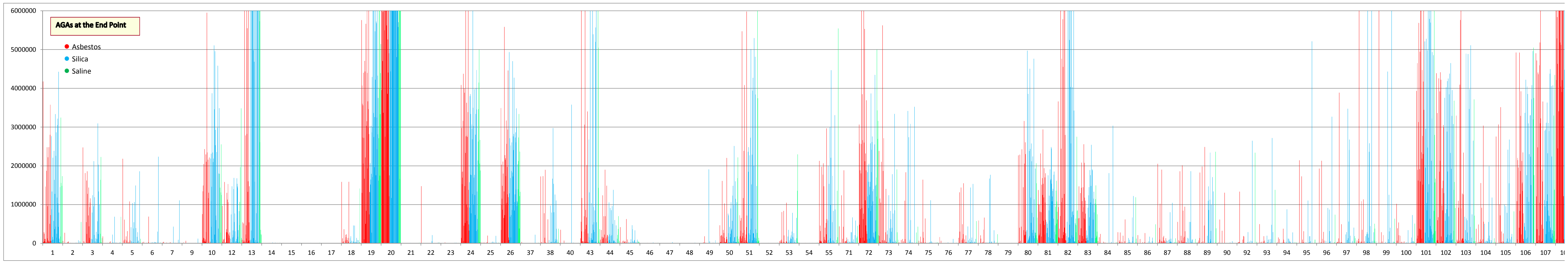
11	65	Asbestos	Humane Endpoint	160 g	Yes	1. One Large Pale Tumor on Liver (2.5 cm, plum shaped) Surrounded by smaller (2mm) yellowish tumors. 2. Intestine impacted by meso, has fewer large tumors over tract 3. Stomach membrane highly perforated + liquid release upon pressure 4. Meso contained within perionteum 5. Ascitic Fluid: 17mLs 6. GI Tract Adhered togheter, Diaphram-Liver feels hard, Liver-Stomach 7. Kidneys reddish and shrunk
12	68	Asbestos	Humane Endpoint	336.0 g	Yes	1. Miliary (and slightly larger 1-2mm) all over abdominal cavity and organs 2. Liver/stomach/diaphram/spleen adhesion
13	12	Asbestos	Study Endpoint	232.0 g	No	1. Negative for meso 2. no ascitic fluid
14	33	Asbestos	Study Endpoint	233.3 g	No	1. Negative for meso 2. no ascitic fluid
15	52	Asbestos	Study Endpoint	220.6 g	No	1. No tumors
16	63	Asbestos	Study Endpoint	210 g	No	1. Negative for meso 2. no ascitic fluid
17	67	Asbestos	Study Endpoint	213.2 g	No	1. Negative for meso 2. no ascitic fluid
18	69	Asbestos	Study Endpoint	236.1 g	No	1. No tumors
19	70	Asbestos	Study Endpoint	217 g	No	1. no tumors
20	4	Asbestos	Study Endpoint	230.3 g	Minimal	1. Overall: minimal tumors 2. tumor between stomach/spleen 3. No discernable milliary spread 4. Beginning of tumor: left retroperitoneum- still red 5. no ascitic fluid
21	58	Asbestos	Study Endpoint	236.3 g	Minimal	1. No visible indication of meso 2. Dipahram/Liver adhesion in Right Upper Quad 3. No ascitic fluid
22	61	Asbestos	Study Endpoint	261.4 g	Minimal	1. Minimal Burden of Disease 2. Some meso beginign to form around retroperitoneum
23	62	Asbestos	Study Endpoint	257.5 g	Minimal	1. Very few (<10) milliary tumors 2. Spleen adhered to organs
24	14	Asbestos	Study Endpoint	201.4 g	Minimal	1. No tumors
25	18	Asbestos	Study Endpoint	215.1 g	Yes	1. Small milliary tumors 2. a lot of free-floating tumors (early) 3. possible ascitic fluid
26	20	Asbestos	Study Endpoint	273.2 g	Yes	1. Free floating tumors 2. lots of ascitic fluid collected
27	21	Asbestos	Study Endpoint	180 g	Yes	1. Miliary tumors spread over abdomen 2. 3cm x 2 cm mass in right lower quadrant 3. 1 large cyst in right lower quadrant
28	51	Asbestos	Study Endpoint	230.3 g	Minimal	1. Extremely early meso 2. Sub-milliary tumors in retroperitoneum (too small to collect) 3. Surface of liver has tiny dots-early tumors? 4. Fat is "struck" to rogans causing shortening 5. blood in abdomen-->not from ascitic fluid, from trauma or Heart Punch
29	53	Asbestos	Study Endpoint	219.4 g	yes	1. Milliary tumors
30	56	Asbestos	Study Endpoint	217.1 g	Yes	1. Tumor inbetween bowls 2. Bowls encased in thin tumor 3. large nodule under liver 4. cyst in retroperitoneum
31	71	Asbestos	Study Endpoint	245.4 g	Yes	1. Tumors all over abdomen 2. Large Tumor: subdiaphramatic on Right side
32	72	Asbestos	Study Endpoint	219 g	Yes	1. Large tumor on Stomach/Liver 2. Tumor under Diaphram 3. over 65mL of ascitic fluid collected
Single Bolus Intraperitoneal Silica Injection						
33	1	Silica Dioxide	Study Endpoint	211.3 g	No	1. 20mL of PBS in abdominal cavity
34	2	Silica Dioxide	Study Endpoint	216.4 g	No	1. 20mL of PBS in abdominal cavity
35	5	Silica Dioxide	Study Endpoint	218.3 g	No	1. 20mL of PBS in abdominal cavity
36	6	Silica Dioxide	Study Endpoint	230.4 g	No	1. 20mL of PBS in abdominal cavity

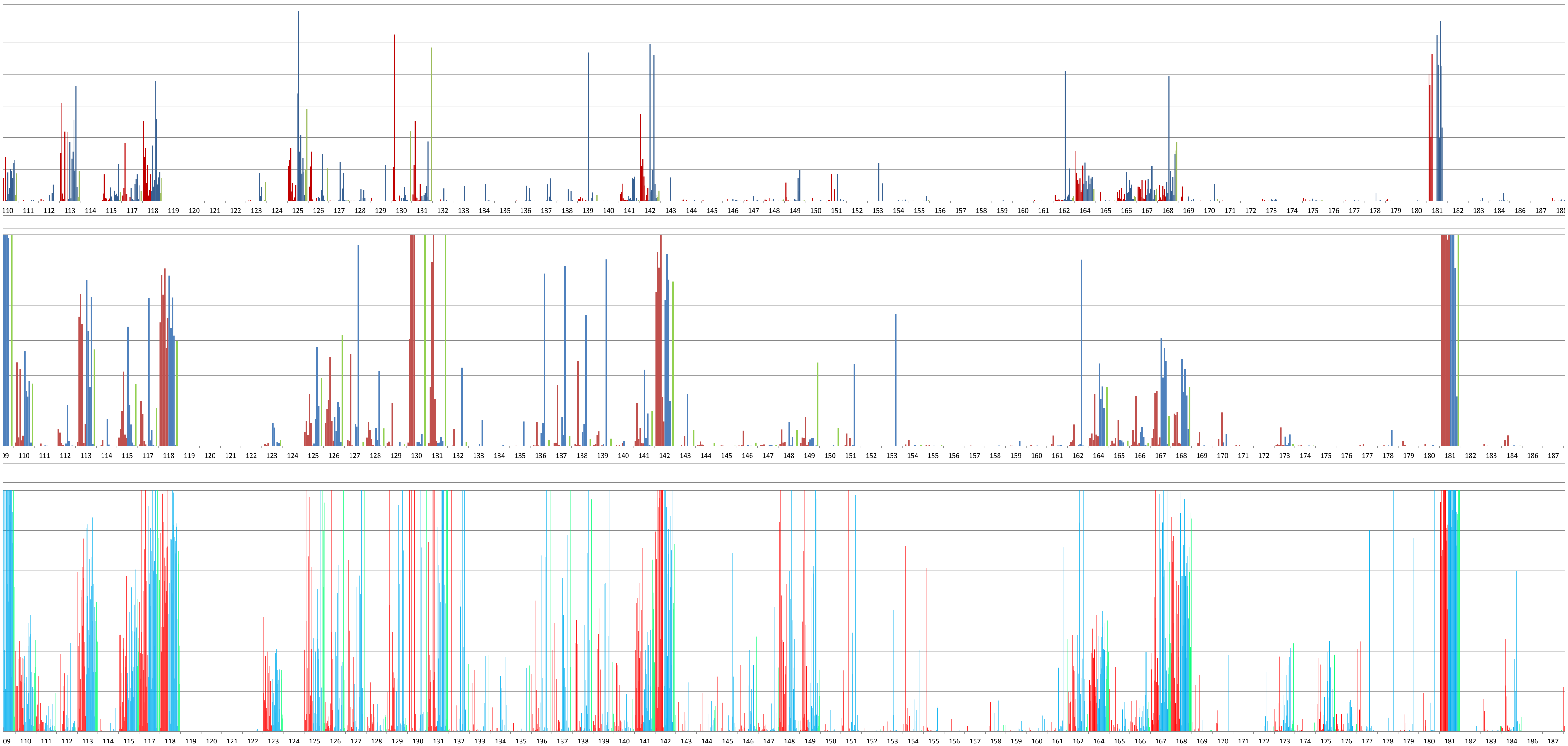
37	7	Silica Dioxide	Study Endpoint	248.5 g	No	1. 20mL of PBS in abdominal cavity
38	8	Silica Dioxide	Study Endpoint	218.2 g	No	1. 20mL of PBS in abdominal cavity
39	9	Silica Dioxide	Study Endpoint	211.4 g	No	1. 20mL of PBS in abdominal cavity
40	10	Silica Dioxide	Study Endpoint	222.2 g	No	1. 20mL of PBS in abdominal cavity
41	11	Silica Dioxide	Study Endpoint	225.5 g	No	1. 20mL of PBS in abdominal cavity
42	13	Silica Dioxide	Study Endpoint	228.4 g	No	1. 20mL of PBS in abdominal cavity
43	15	Silica Dioxide	Study Endpoint	203.1 g	No	1. 20mL of PBS in abdominal cavity
44	17	Silica Dioxide	Study Endpoint	222.1 g	No	1. 20mL of PBS in abdominal cavity
45	19	Silica Dioxide	Study Endpoint	217.7 g	No	1. 20mL of PBS in abdominal cavity
46	22	Silica Dioxide	Study Endpoint	233.5 g	No	1. 20mL of PBS in abdominal cavity
47	23	Silica Dioxide	Study Endpoint	222.1 g	No	1. 20mL of PBS in abdominal cavity
48	24	Silica Dioxide	Study Endpoint	228.3 g	No	1. 20mL of PBS in abdominal cavity
49	25	Silica Dioxide	Study Endpoint	201.7 g	No	1. 20mL of PBS in abdominal cavity
50	26	Silica Dioxide	Accidental Death - heart attack	x	No	
51	28	Silica Dioxide	Study Endpoint	228.6 g	No	1. 20mL of PBS in abdominal cavity
52	29	Silica Dioxide	Study Endpoint	234 g	No	1. 20mL of PBS in abdominal cavity
53	30	Silica Dioxide	Study Endpoint	225.5 g	No	1. 20mL of PBS in abdominal cavity
54	32	Silica Dioxide	Study Endpoint	243.8 g	No Meso, Sarcoma found	1. Sarcoma- natural and spontaneous 2. Mass coming from the fascia of the muscle 3. like "fish flesh" 4. normal abdomen
55	35	Silica Dioxide	Study Endpoint	231 g	No	1. 20mL of PBS in abdominal cavity
56	38	Silica Dioxide	Study Endpoint	216.9 g	No	1. 20mL of PBS in abdominal cavity
57	40	Silica Dioxide	Study Endpoint	245.1 g	No	1. 20mL of PBS in abdominal cavity
58	41	Silica Dioxide	Study Endpoint	225.3 g	No	1. 30mL of PBS in abdominal cavity
59	42	Silica Dioxide	Study Endpoint	218.3 g	No	1. 20mL of PBS in abdominal cavity
60	44	Silica Dioxide	Study Endpoint	225.5 g	No	1. 20mL of PBS in abdominal cavity
61	45	Silica Dioxide	Study Endpoint	205.8 g	No	1. 20mL of PBS in abdominal cavity
62	47	Silica Dioxide	Study Endpoint	242.8 g	No	1. 20mL of PBS in abdominal cavity
63	48	Silica Dioxide	Study Endpoint	226.5 g	No	1. 20mL of PBS in abdominal cavity
64	50	Silica Dioxide	Study Endpoint	226.7 g	No	1. 20mL of PBS in abdominal cavity
Single Intraperitoneal Saline Injection						
65	27	Saline	Study Endpoint	231.1 g	No	1. 20mL of PBS in abdominal cavity
66	31	Saline	Study Endpoint	234.4 g	No	1. 20mL of PBS in abdominal cavity
67	34	Saline	Study Endpoint	231 g	No	1. 20mL of PBS in abdominal cavity
68	36	Saline	Study Endpoint	212.9 g	No	1. 20mL of PBS in abdominal cavity
69	37	Saline	Study Endpoint	229.3 g	No	1. 20mL of PBS in abdominal cavity
70	39	Saline	Study Endpoint	211.5 g	No	1. 20mL of PBS in abdominal cavity
71	46	Saline	Study Endpoint	226.3 g	No	1. 20mL of PBS in abdominal cavity
72	49	Saline	Study Endpoint	233.3 g	No	1. 20mL of PBS in abdominal cavity

Award Number: W81XWH-10-0399 and W81XWH-10-400
TITLE: Glyco-Immune Diagnostic Signatures and Therapeutic Targets of Mesothelioma
PRINCIPAL INVESTIGATORS: Harvey Pass, MD; Margaret Huflejt, PhD
New York University School of Medicine
Attachment 2 of 4





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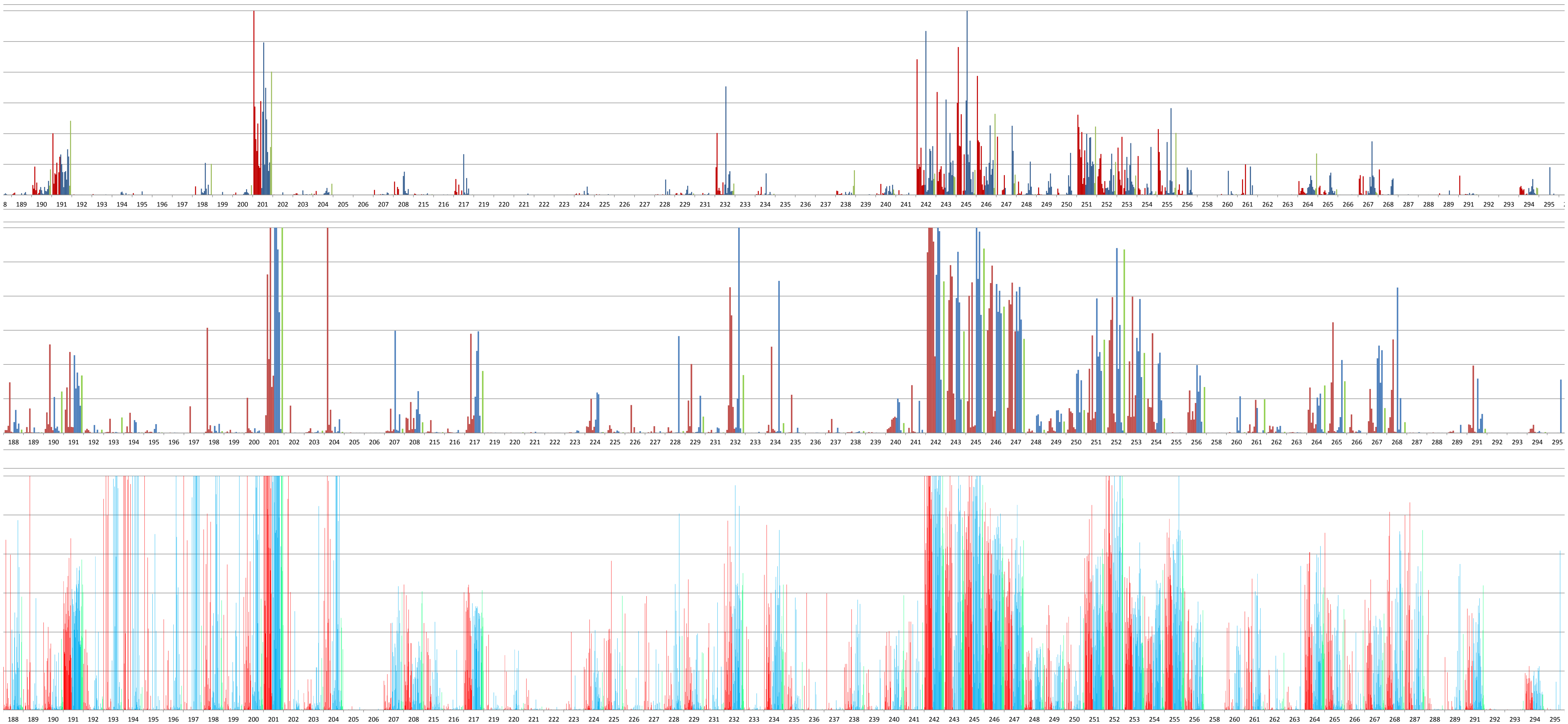
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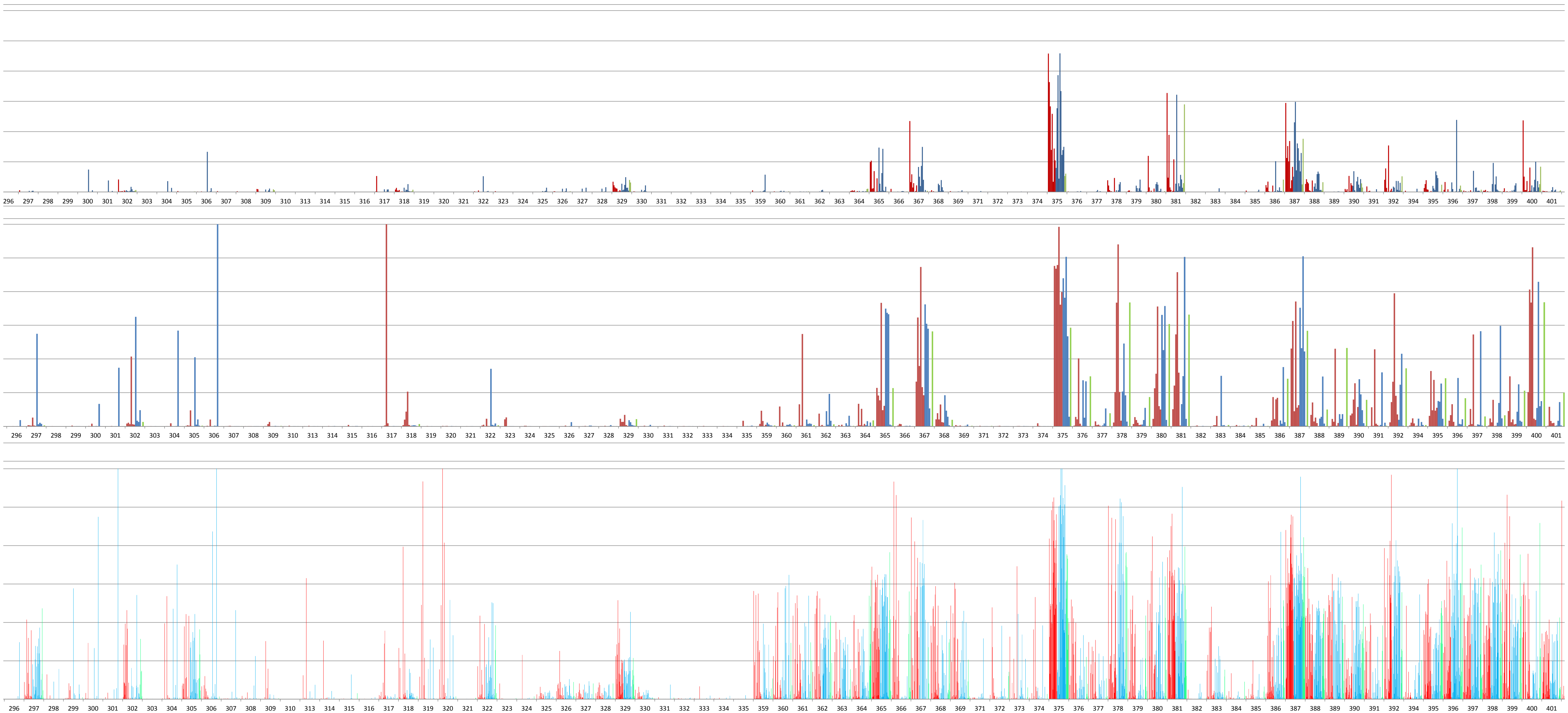
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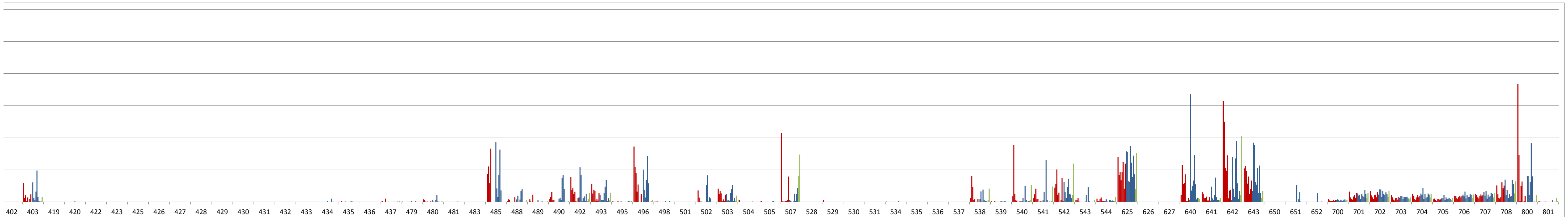
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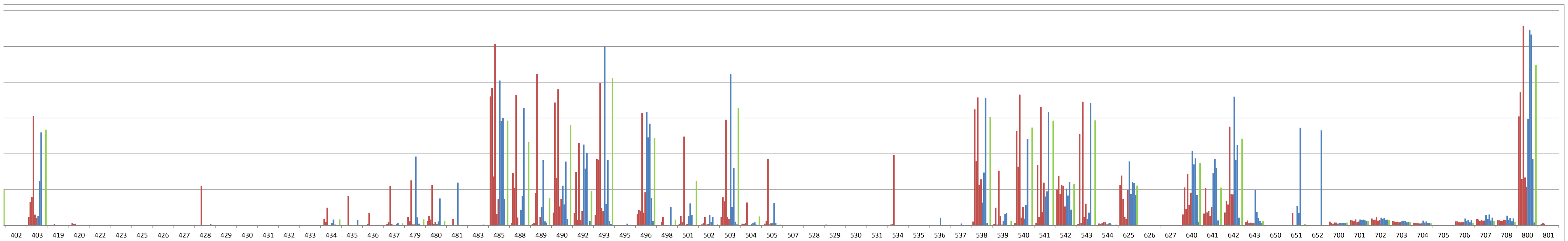
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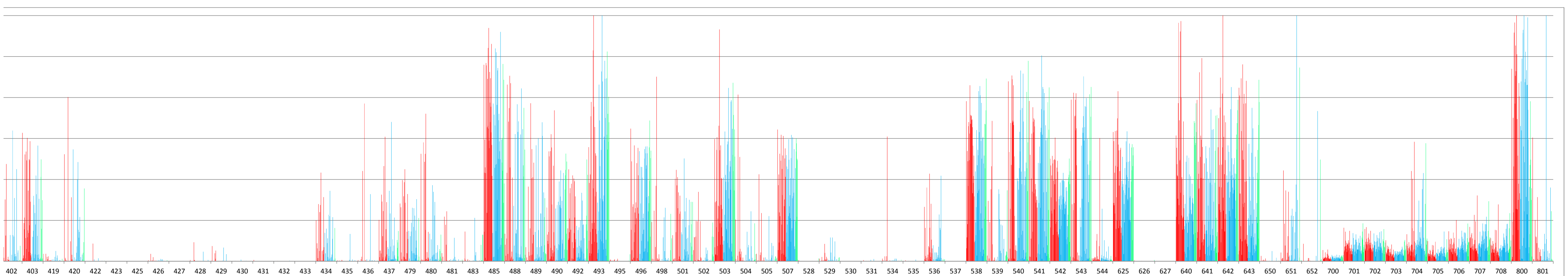
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TITLE: Glyco-Immune Diagnostic Signatures and Therapeutic Targets of Mesothelioma

PRINCIPAL INVESTIGATORS: Harvey Pass, MD; Margaret Huflejt, PhD

New York University School of Medicine

Attachment 3 of 4

Dynamics of Anti-Glycan Antibodies (AGAs) Observed Four Weeks Following Injection of Asbestos or Silica

GID: Glycan Identification number

X = Antibodies against this glycan show significant dynamics in human populations of asbestos-exposed subjects and patients with Malignant Mesothelioma

GID	Structure	Additional Comments
Increase in AGA Signals Four Weeks Post-Injection in Response to Asbestos		
112	**Glcβ1-6Glcβ-sp4**	X; Appearance in Asbestos rats
117	**GlcNAcβ1-4GlcNAcβ-sp4**	Slight decrease in Silica rats
128	**Galβ1-4Glcβ-sp4-Trp**	X; Appearance in Asbestos rats
130	**(6-O-Bn-Galβ1)-3GlcNAcβ-sp3**	X; Significant increase in Asbestos rats
131	**(6-O-Bn-Galβ1)-3(6-O-Bn)GlcNAcβ-sp3**	X; Significant increase in Asbestos rats
149	**GlcNAcβ1-4(6-O-Su)GlcNAcβ-sp2**	X; Appearance in Asbestos rats
204	**4-O-Su-GalNAcβ1-4GlcNAcβ-sp2**	Appearance in Asbestos rats
268	**GlcNAcβ1-4(Fucα1-6)GlcNAcβ-sp3**	Appearance in Asbestos rats
318	**Neu5Acα2-6Galβ1-4-(6-O-Su)GlcNAcβ-sp3**	Low signals, appearance in some rats, increase in others
368	**GalNAcα1-3** **Galβ1-4GlcNAcβ-sp3** **Fucα1-2**	X; Appearance of Asbestos rats
400	**Galβ1-3GlcNAcα1-3Galβ1-3GlcNAcβ-sp3**	X; Slight increase in Silica rats but not significant
488	**Galβ1-4GlcNAcβ1-6** **GalNAcα-sp3** **Galβ1-4GlcNAcβ1-3**	Appearance in Asbestos rats
490	**Galβ1-4GlcNAcβ1-6** **Galβ1-4GlcNAcβ-sp2** **GlcNAcβ1-3**	Significant increase in Asbestos rats; slight increase in Silica rats but not significant
540	**Le^{x}1-6'(6'SLN1-3')Lac-sp4**	
Increase in AGA Signals Four Weeks Post-Injection in Response to Silica		
24	**GlcNAcα-sp3**	Decrease in Asbestos rats
26	**Rhaβ-sp4**	
83	**Galα1-6Glcβ-sp4**	Some Asbestos rats exhibited increased signals
101	**GalNAcα1-3GalNAcβ-sp3**	
102	**GalNAcα1-3Galβ-sp3**	Appearance in Silica rats
106	**GalNAcβ1-4GlcNAcβ-sp3**	X
107	**GalNAcβ1-4GlcNAcβ-sp2**	X
110	**Glcα1-4Glcβ-sp3**	Small decrease in Asbestos rats
164	**GlcAβ1-3GlcNAcβ-sp3**	X; Decrease in Asbestos rats
191	**6-P-Galβ1-4GlcNAcβ-sp2**	Decrease in Asbestos rats
201	**3,4-O-Su$_2$-GalNAcβ1-4-GlcNAcβ-sp3**	Large increase in Silica rats
245	**GlcNAcα1-6Galβ1-4GlcNAcβ-sp2**	X
251	**GlcNAcβ1-4Galβ1-4GlcNAcβ-sp2**	X; decrease in Asbestos rats
252	**GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ-sp4**	X
253	**GlcNAcβ1-6Galβ1-4GlcNAcβ-sp2**	
387	**Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAcβ-sp2**	Decrease in Asbestos rats
396	**(GlcNAcβ1)$_3$-3,4,6-GalNAcα-sp3**	Decrease in Asbestos rats
Shared Increases in AGA Signals within Asbestos Injected and Silica Injected Rats		
19	**ManNAcβ-sp4**	
20	**Rhaα-sp3**	Slightly larger increase in Silica rats than Asbestos rats
72	**Fucα1-3GlcNAcβ-sp3**	
82	**Galα1-4GlcNAcβ-sp8**	
109	**GalNAc$_{(furanose)}$β1-4GlcNAcβ-sp2**	
113	**GlcNAcβ1-3GalNAcα-sp3**	
115	**GlcNAcβ1-4GlcNAcβ-Asn**	Greater increase in Asbestos rats compared to Silica rats
118	**GlcNAcβ1-6GalNAcα-sp3**	
126	**6-Bn-Galα1-4(6-Bn)GlcNAcβ-sp**	X; Greater increase in Asbestos rats
142	**GlcNAcα1-3GalNAcβ-sp3**	Large increases in both Asbestos rats and Silica rats
167	**GlcNAcβ1-4-[HOOC(CH$_3$)CH]-3-O-GlcNAcβ-sp4**	X; Greater in Silica rats
168	**GlcNAcβ1-4Mur-L-Ala-D-i-Gln-Lys**	Greater in Silica rats

181	**3,4-O-Su$_2$-Galβ1-4GlcNAcβ-sp3**	Significant increase in Silica rats
194	**6-O-Su-GalNAcβ1-4GlcNAcβ-sp3**	Appearance in both Asbestos rats and Silica rats
217	**Fucα1-2Galβ1-3GalNAcα-sp3**	
224	**Galα1-4Galβ1-4Glcβ-sp3**	Greater in Silica rats
232	**Galβ1-4GlcNAcβ1-6GalNAcα–sp3**	Appearance in Asbestos rats
242	**GlcNAcα1-3Galβ1-4GlcNAcβ-sp2**	**X**; more significant in Asbestos rats compared to Silica rats
243	**GlcNAcα1-3Galβ1-4GlcNAcβ-sp3**	
246	**GlcNAcβ1-2Galβ1-3GalNAcα-sp3**	
247	**GlcNAcβ1-3Galβ1-3GalNAcα-sp3**	
250	**GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3**	Appearance in Asbestos rats; increased signals in Silica rats
254	**GlcNAcβ1-6** **GalNAcα-sp3** **Galβ1-3**	More significant in Asbestos rats
256	**GlcNAcβ1-6** **GalNAcα-sp3** **GlcNAcβ1-4**	Appearance of Asbestos rats, increase in Silica rats
264	**Galβ1-4Galβ1-4GlcNAcβ-sp3**	
267	**GlcNAcβ1-3Galβ1-3GlcNAcβ-sp3**	
365	**Galα1-4** **Galβ1-4GlcNAcβ-sp3** **Fucα1-2**	Greater in Silica rats
367	**GalNAcα1-3** **Galβ1-4GlcNAcβ-sp2** **Fucα1-2**	
375	**Galα1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3**	Large increase in both Asbestos rats and Silica rats
376	**Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-sp4**	Appearance in both Asbestos rats and Silica rats
378	**Galβ1-3GlcNAcα1-3Galβ1-4GlcNAcβ-sp3**	
380	**Galβ1-3GlcNAcα1-6Galβ1-4GlcNAcβ-sp2**	Slightly larger increase in Silica rats than Asbestos rats
381	**Galβ1-3GlcNAcβ1-6Galβ1-4GlcNAcβ-sp2**	Slightly larger increase in Asbestos rats than Silica rats
389	**GalNAcβ1-3Galα1-4Galβ1-4Glcβ-sp3**	Appearance of both Asbestos rats and Silica rats
390	**(Glcα1-4)$_4$β-sp4**	
392	**GalNAcα1-3** **Galβ1-3GalNAcα-sp3** **Fucα1-2**	
395	**GlcNAcβ1-6** **Galβ1-4GlcNAcβ-sp2** **GlcNAcβ1-3**	
403	**Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-sp2**	
485	**Galβ1-4GalNAcα1-3** **Galβ1-4GlcNAcβ-sp3** **Fucα1-2**	
489	**GlcNAcβ1-6** **Galβ1-4GlcNAc-sp2** **Galβ1-4GlcNAcβ1-3**	
493	**(GlcNAcβ1-4)$_5$β-sp4**	
538	**Le^{x}1-6'(Le^{C}1-3')Lac-sp4**	More significant in Asbestos rats compared to Silica rats
542	**Le^{C}Le^{x}1-6'(Le^{C}1-3')Lac-sp4**	
543	**Le^{x}1-6'(Le^{B}1-3')Lac-sp4**	
641	**E.coli oligosaccharide -2 (1208)**	
642	**E.coli oligosaccharide -3 (1210)**	

Award Number: W81XWH-10-0399 and W81XWH-10-400

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New York University School of Medicine

Attachment 4 of 4

Cage	Rat	6/13/2014	6/27/2014	7/1/2014	7/11/2014	7/15/2014		7/18/2014
		Day - 18	Day - 4	Day 0	Day 10	Day 14		Day 17
		Weight (g)	Weight (g)	Injection	DLAR Notes	Weight (g)	Tumor Measurment (cm) and Notes	GT Notes
872785	45	120	146	Saline SC		158.7		Selected for Necropsy
	30	100	138	Saline SC		156.5		Selected for Necropsy
872786	38	100	147	Saline SC		162.3		Selected for Necropsy
	39	110	145	Saline SC		161.6		
872804	42	120	145	Saline SC		165.2		
	46	120	150	Saline SC		165.6		
872803	41	100	138	Saline IP		155.5		Selected for Necropsy
	44	100	130	Saline IP		148.4		Selected for Necropsy
872788	47	120	150	Saline IP		166.8		Selected for Necropsy
	43	120	147	Saline IP		167.0		
872787	40	100	139	Saline IP		158.8		
	48	120	147	Saline IP		164.8		
872782	21	120	155	Cell Lines SC		176.2	0.71 X 0.88	Selected for Necropsy
	15	100	144	Cell Lines SC		163.2	1.05 X 1.04	Selected for Necropsy
872790	24	100	144	Cell Lines SC		162.5	0.45 X 0.45	
	10	100	140	Cell Lines SC		154.3	small scattered, about .5 X.3 each	
872784	14	100	139	Cell Lines SC		162.3	2 small grains	
	13	120	146	Cell Lines SC		164.2	1 or 2 small scattered	
872805	20	120	146	Cell Lines SC		164.0	estimated 1.0 X 0.6 oblong shape	Selected for Necropsy
	23	120	150	Cell Lines SC		170.4	2 small scattered grains, estimated .3 X .4 each	
872783	18	120	148	Cell Lines SC		162.4	1.1 X 0.8 - oblong shape	
	22	120	150	Cell Lines SC		168.0	4 large grains	
872798	17	120	149	Cell Lines SC		162.0	4 medium grains	
	16	120	153	Cell Lines SC		167.6	1 small, 3 medium grains	Monitor tumor surface
872789	7	120	149	Cell Lines IP		170.7	1 small, 1 medium grain	1 cm oblong tumor
	9	120	142	Cell Lines IP		162.0	0.6 X 0.7	1 cm oblong tumor
872797	2	140	144	Cell Lines IP		163.2	1 small, 1 medium grain	
	8	120	152	Cell Lines IP		173.8	2 medium grains - one on top of the other	Selected for Necropsy
872796	19	100	139	Cell Lines IP		156.3	2 med-small grains	Selected for Necropsy
	1	120	146	Cell Lines IP		165.2	1 small grain	Tumor almost not palpable
872791	6	100	144	Cell Lines IP		167.4	2 medium grains	
	3	120	152	Cell Lines IP		165.3	1 medium grain	
872794	28	120	149	Cell Lines IP		172.1	1 large, 1 small grain	Selected for Necropsy
	29	120	149	Cell Lines IP		179.0	No palpable tumors	No palpable tumors
872795	27	100	149	Cell Lines IP		163.2	1 small, 1 medium oblong-shaped grain	
	35	100	147	Cell Lines IP		168.7	2 medium grains - one on top of the other	
872793	11	100	151	Tail Vein (.18 mL)	Deceased (7/7/14)	X	X	X
	4	120	152	Tail Vein (.20 mL)		158.4		Selected for Necropsy
872792	5	100	145	Tail Vein (.20 mL)	Healthy, but wt. loss (140g)	157.1		Selected for Necropsy
	12	110	147	Tail Vein (.20 mL)	Thin (100 g), wet genital opening, small chin wound	139.2		Selected for Necropsy
872801	31	120	148	Tail Vein (.20 mL)	Eye crust, dried porphyrin, wt. loss (120 g)	151.3		
	33	120	141	Tail Vein (.20 mL)	Wt. loss (120 g)	156.2		
872800	26	110	147	Tail Vein (.14 mL)		163.2		
	36	120	151	Tail Vein (.20 mL)		168.6		
872799	37	100	144	Tail Vein (.18 mL)		162.7		
	25	120	143	Tail Vein (.20 mL)		158.6		
872802	34	120	149	Tail Vein (.20 mL)		161.2		
	32	100	142	Tail Vein (.20 mL)		160.5		

Cage	Rats	Treatment	7/23/2014		7/24/2014		7/25/2014	
			Day 22		Day 23		Day 24	
			Notes		Weight (g)	Necropsy Notes	Weight (g)	Tumor Measurement (cm) & Notes
872785	45	Saline SC		Selected for necropsy	165.2		X	X
	30	Saline SC			160.7		X	X
872786	38	Saline SC		Selected for necropsy	161.9	1. Normal coloration	X	X
	47	Saline IP	Used to be in cage 788		170.4	1. Horizontal incision to show natural colors of control animal organs and natural pleural cavity architecture	X	X
872804	42	Saline SC					169.2	
	46	Saline SC					170.6	
872803	41	Saline IP		Selected for necropsy	157.5		X	X
	44	Saline IP			153.7		X	X
872788	39	Saline SC	Used to be in cage 786	New mixed cage			161.9	
	43	Saline IP					174.3	
872787	40	Saline IP					167.7	
	48	Saline IP					171.5	
872782	21	Cell Lines SC		Selected for necropsy	181.2		X	X
	15	Cell Lines SC			177.5	1. Minimal liver discoloration	X	X
872790	24	Cell Lines SC					177.6	1 large oblong tumor ≈ 2 X 1
	10	Cell Lines SC					164.1	1 large tumor ≈ 1.5 X 1.5 & 2 med grains
872784	14	Cell Lines SC					170.4	2 large tumors; 1 ≈ 2.5 X 1
	13	Cell Lines SC					173.8	2.5 X 1
872805	20	Cell Lines SC		Selected for necropsy	170.6	1. Large tumor removed from scapula 2. "Very hard tumor that grows but does not kill" (MEH) 3. Normal internal coloration and few tumors on mesotheric membrane	X	X
	28	Cell Lines IP	Used to be in cage 794		171.5		X	X
872783	18	Cell Lines SC					175.4	1 big, hard cluster ≈ 2.5 X 2
	22	Cell Lines SC					176.9	2 large tumors; 1 ≈ 2 X 1
872798	17	Cell Lines SC					171.1	1 large oblong tumor ≈ 2 cm in length
	16	Cell Lines SC					181.6	1 large oblong tumor ≈ 2.5 cm in length
872789	7	Cell Lines IP		Deceseased as of 7/23/14	X	X	X	X
	9	Cell Lines IP					167.2	1 large tumor ≈ 1.5 X 1
872797	19	Cell Lines IP	Used to be in Cage 796	Selected for necropsy	163.9	1. Tumors in pleural cavity 2. Large tumor in lower abdomen	X	X
	8	Cell Lines IP			171.3	1. Stomach covered will milliary tumors 2. Liver only slightly discolored 3. Tumors taking over sternum 4. Tumors all over peritoneal cavity 5. Diaphram thickened and distorted	X	X
872796	2	Cell Lines IP	Used to be in cage 797	Deceseased as of 7/23/14	X	X	X	X
	1	Cell Lines IP					166.4	few small abdominal grains - *moved to cage #872789 taking deceased 7's spot
872791	6	Cell Lines IP		Selected for necropsy 6: hunched, ruffled, and a little pale. The rat was moving around the cage when opened, but moving about slowly and returned to a still position when placed on the rack. The rat was seen eating moistened food I placed on the cage floor. It was not interested in the diet-gel cup. (Heather DiPietro)	Deceased as of 7/23/14	1. Lots of miliary tumors and disoloration 2. Tumors on intestine 3. Peritoneal is covered in miliary tumors	X	X
	27	Cell Lines IP			141	1. Yellow discoloration 2. Tumors beneath intestines and upper chest cavity 3. Tumors lining liver	X	X
872794	23	Cell Lines SC	Used to be in cage 805	New mixed cage			174.5	1 med-large, hard tumor ≈ 1 X 0.8
	29	Cell Lines IP					182.0	no palpable tumors
872795	3	Cell Lines IP	Used to be in cage 791				164.8	1 med tumor ≈ 0.8 X 0.7
	35	Cell Lines IP					164.6	1 med-large tumor ≈ 1.2 X 0.8
872793	11	Gemzar Tail Vein	X	X	X	X	X	X
	4	Gemzar Tail Vein		Selected for necropsy			X	X
872792	5	Gemzar Tail Vein		Selected for necropsy		1. Normal colored spleaen	X	X
	12	Gemzar Tail Vein				1. Large stomach that is hard to the touch (excised)	X	X
872801	31	Gemzar Tail Vein					180.0	
	33	Gemzar Tail Vein					160.1	
872800	26	Gemzar Tail Vein					177.0	
	36	Gemzar Tail Vein					174.1	
872799	37	Gemzar Tail Vein					174.3	
	25	Gemzar Tail Vein					173.6	
872802	34	Gemzar Tail Vein					173.3	
	32	Gemzar Tail Vein					168.9	

Cage	Rats	7/29/2014		7/31/2014	
		Day 28		Day 30	
		Weight (g)	Necropsy Notes	Weight (g)	Necropsy Notes
872785	45	X	X	X	X
	30	X	X	X	X
872786	38	X	X	X	X
	47	X	X	X	X
872804	42			170.4	1. Normal coloration 2. No visible tumors
	46			171.1	1. Normal coloration 2. No visible tumors
872803	41	X	X	X	X
	44	X	X	X	X
872788	39			166.4	1. Normal coloration 2. No visible tumors
	43			178.1	1. Normal coloration 2. No visible tumors
872787	40			172	1. Normal coloration 2. No visible tumors
	48			170.8	1. Normal coloration 2. No visible tumors
872782	21	X	X	X	X
	15	X	X	X	X
872790	24	180.1	1. Normal abdominal and thoracic cavity	X	X
	10	170,6	1. Normal abdominal and thoracic cavity 2. Tumor in scapula broke through skin	X	X
872784	14	171.9	1. Normal abdominal and thoracic cavity	X	X
	13	180.3	1. Normal abdominal and thoracic cavity 2. Tumor in scapula adhering to outside of skull	X	X
872805	20	X	X	X	X
	28	X	X	X	X
872783	18	184.4	1. Normal abdominal and thoracic cavity	X	X
	22	181.2	1. Normal abdominal and thoracic cavity	X	X
872798	17	174.5	1. Normal abdominal and thoracic cavity	X	X
	16	187.3	1. Normal abdominal and thoracic cavity	X	X
872789	1	152	1. Large tumor on sternum and abdominal wall 2. Sizabletumors filling abdominal cavity and covering intestine 3. Scattered miliary tumors throughout abdominal cavity 4. No tumors visible in thoracic cavity	X	X
	9	172.5	1. Tumor on site of injection (~ 2 cm diameter) 2. Multiple tumors on sternum 3. Miliary tumors covering small intestine	X	X
872797	19	X	X	X	X
	8	X	X	X	X
872796	2	X	X	X	X
		Rat 1 used to be here. Transferred to 789		X	X
872791	6	X	X	X	X
	27	X	X	X	X
872794	23	177.4	1. Normal abdominal and thoracic cavity	X	X
	29	184.2	1. Normal coloration and no discernable tumors	X	X
872795	3	Deceased as of 7/28/14 (post-mortem necropsy)	1. Pale miliary tumors all over intestinal lining and messenteric fat 2. Tumors on spleen 3. Discoloration of liver (pale) 4. Normal lungs and heart	X	X
	35	151.5	1. Discoloration of internal organs 2. Thoracic cavity filled with tumors 3. Tumor adhering to sternum 4. Tumors attached to mesenteric membrane and spleen	X	X
872793	11	X	X	X	X
	4	X	X	X	X
872792	5	X	X	X	X
	12	X	X	X	X
872801	31			185.7	1. Normal coloration 2. No visible tumors
	33			163	1. Normal coloration 2. No visible tumors
872800	26			178.6	1. Normal coloration 2. No visible tumors
	36			174.4	1. Normal coloration 2. No visible tumors
872799	37			175.3	1. Normal coloration 2. No visible tumors
	25			174.4	1. Normal coloration 2. No visible tumors
872802	34			175.7	1. Normal coloration 2. No visible tumors
	32			171.8	1. Normal coloration 2. No visible tumors

Rat	6/13/2014	6/27/2014	7/1/2014	7/11/2014	7/15/2014	7/24/2014	7/25/2014	7/29/2014	7/31/2014	
	Day - 18	Day - 4	Day 0	Day 13	Day 14	Day 23	Day 24	Day 28	Day 30	
	Weight (g)	Weight (g)	Injection	DLAR notes	Weight (g)	Weight (g)	Weight (g)	Weight (g)	Weight (g)	Endpoint
45	120	146	Saline SC (.20 mL)		158.7	165.2 (Necropsy)	X	X	X	Study Endpoint
30	100	138	Saline SC (.20 mL)		156.5	160.7 (Necropsy)	X	X	X	Study Endpoint
38	100	147	Saline SC (.20 mL)		162.3	161.9 (Necropsy)	X	X	X	Study Endpoint
39	110	145	Saline SC (.20 mL)		161.6		161.9		166.4 (Necropsy)	Study Endpoint
42	120	145	Saline SC (.20 mL)		165.2		169.2		170.4 (Necropsy)	Study Endpoint
46	120	150	Saline SC (.20 mL)		165.6		170.6		171.1 (Necropsy)	Study Endpoint
41	100	138	Saline IP (.20 mL)		155.5	157.5 (Necropsy)	X	X	X	Study Endpoint
44	100	130	Saline IP (.20 mL)		148.4	153.7 (Necropsy)	X	X	X	Study Endpoint
47	120	150	Saline IP (.20 mL)		166.8	170.4 (Necropsy)	X	X	X	Study Endpoint
43	120	147	Saline IP (.20 mL)		167.0		174.3		178.1 (Necropsy)	Study Endpoint
40	100	139	Saline IP (.20 mL)		158.8		167.7		172 (Necropsy)	Study Endpoint
48	120	147	Saline IP (.20 mL)		164.8		171.5		170.8 (Necropsy)	Study Endpoint
21	120	155	Cell Line SC (.20 mL)		176.2	181.2 (Necropsy)	X	X	X	Humane Endpoint
15	100	144	Cell Line SC (.20 mL)		163.2	177.5 Necropsy)	X	X	X	Humane Endpoint
24	100	144	Cell Line SC (.20 mL)		162.5		177.6	180.1 (Necropsy)	X	Study Endpoint
10	100	140	Cell Line SC (.20 mL)		154.3		164.1	170.6 (Necropsy)	X	Study Endpoint
14	100	139	Cell Line SC (.20 mL)		162.3		170.4	171.9 (Necropsy)	X	Study Endpoint
13	120	146	Cell Line SC (.20 mL)		164.2		173.8	180.3 (Necropsy)	X	Study Endpoint
20	120	146	Cell Line SC (.20 mL)		164.0	170.6 Necropsy)	X	X	X	Humane Endpoint
23	120	150	Cell Line SC (.20 mL)		170.4		174.5	177.4 (Necropsy)	X	Study Endpoint
18	120	148	Cell Line SC (.20 mL)		162.4		175.4	184.4 (Necropsy)	X	Study Endpoint
22	120	150	Cell Line SC (.20 mL)		168.0		176.9	181.2 (Necropsy)	X	Study Endpoint
17	120	149	Cell Line SC (.20 mL)		162.0		171.1	174.5 (Necropsy)	X	Study Endpoint
16	120	153	Cell Line SC (.20 mL)		167.6		181.6	187.3 (Necropsy)	X	Study Endpoint
7	120	149	Cell Lines IP (.20 mL)		170.7	Deceseased (7/23/14)	X	X	X	Found Dead
9	120	142	Cell Lines IP (.20 mL)		162.0		167.7	172.5 (Necropsy)	X	Study Endpoint
2	140	144	Cell Lines IP (.20 mL)		163.2	Deceseased (7/23/14)	X	X	X	Found Dead
8	120	152	Cell Lines IP (.20 mL)		173.8	171.3 (Necropsy)	X	X	X	Humane Endpoint
19	100	139	Cell Lines IP (.20 mL)		156.3	163.9 (Necropsy)	X	X	X	Humane Endpoint
1	120	146	Cell Lines IP (.20 mL)		165.2		166.4	152 (Necropsy)	X	Study Endpoint
6	100	144	Cell Lines IP (.20 mL)		167.4	Deceased (7/23/14)	X	X	X	Found Dead
3	120	152	Cell Lines IP (.20 mL)		165.3		164.8	Deceased (7/28/14) (post-mortem necropsy)	X	Found Dead
28	120	149	Cell Lines IP (.20 mL)		172.1	171.5 (Necropsy)	X	X	X	Humane Endpoint
29	120	149	Cell Lines IP (.20 mL)		179.0		182	184.2 (Necropsy)	X	Study Endpoint
27	100	149	Cell Lines IP (.20 mL)		163.2	141 (Necropsy)	X	X	X	Humane Endpoint
35	100	147	Cell Lines IP (.20 mL)		168.7		164.6	151.5 (Necropsy)	X	Study Endpoint
11	100	151	Tail Vein (.18 mL)		Deceased (7/7/14)	X	X	X	X	Found Dead
4	120	152	Tail Vein (.20 mL)		158.4	174.8 (Necropsy)	X	X	X	Study Endpoint
5	100	145	Tail Vein (.20 mL)	140	157.1	163.6 (Necropsy)	X	X	X	Study Endpoint
12	110	147	Tail Vein (.20 mL)	100	139.2	169 (Necropsy)	X	X	X	Study Endpoint
31	120	148	Tail Vein (.20 mL)	Dried porphyrin and wt. loss	151.3		180		185.7 (Necropsy)	Study Endpoint
33	120	141	Tail Vein (.20 mL)	120	156.2		160.1		163 (Necropsy)	Study Endpoint
26	110	147	Tail Vein (.14 mL)		163.2		177		178.6 (Necropsy)	Study Endpoint
36	120	151	Tail Vein (.20 mL)		168.6		174.1		174.4 (Necropsy)	Study Endpoint
37	100	144	Tail Vein (.18 mL)		162.7		174.3		175.3 (Necropsy)	Study Endpoint
25	120	143	Tail Vein (.20 mL)		158.6		173.6		174.4 (Necropsy)	Study Endpoint
34	120	149	Tail Vein (.20 mL)		161.2		173.3		175.7 (Necropsy)	Study Endpoint
32	100	142	Tail Vein (.20 mL)		160.5		168.9		171.8 (Necropsy)	Study Endpoint